IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of Aviv Shaish

Serial No. 10/668,601 Group Art Unit: 1654

Filed: 09/24/2003 Examiner: M.C. Flood

For: THERAPEUTIC USES OF DUNALIELLA POWDER

SHAISH II DECLARATION UNDER RULE 132

Commissioner of Patents and Trademarks
Washington, D.C. 20231

I, Aviv Shaish, an Israeli citizen residing at Thalmei Yehyel 200, Israel, hereby declare:

1. I am currently a scientist at the Sheba Medical Center, Israel.

2. My list of publications is attached herewith as **Annex A**. My fields of expertise include carotenoids and atherosclerosis.

3. I am re-submitting the Shaish I Declaration previously submitted on October 4, 2004, in order to correct a clerical error in that declaration, namely the omission of the word "crude" before every instance where the phrase "*Dunaliella* powder" is present.

4. I carried out experiments to test the effect of *Dunaliella* on diabetes. Female db/db mice (n=20) were divided into 2 groups (n=10 in each group). 10 mice were fed chow diet alone (group A) and 10 mice were fed chow diet fortified with crude *Dunaliella* powder (group B) (8% of the diet, weight/weight). After 8 weeks group A was divided into 2 sub-groups:

(a) Control- was continued to be fed chow diet, and

(b) Rosiglitazone- was treated with a low dose of the thiazolidinedione Rosiglitazone (0.005% of the diet, weight/weight).

5. Group B was also divided into 2 sub-groups:

(a) *Dunaliella*- was continued to be fed chow diet with crude Dunaliella powder, and

(b) *Dunaliella* + Rosiglitazone - was treated with crude Dunaliella powder and a low dose of Rosiglitazone.

6. **Results**

In the ***Dunaliella*** **+ Rosiglitazone** treated group, glucose tolerance (area under curve) improved significantly, P=0.005 and P=0.028 as compared to the Control and Rosiglitazone treated groups, respectively (Figs. 1 A,B). Moreover, fasting plasma glucose was lowered in the ***Dunaliella*** **+ Rosiglitazone** group as compared to the other groups (Fig. 2). However, this change did not reach significance.

7. To study the effect of high-dose rosiglitazone plus Dunaliella on diabetes, 40 mice db/db mice were divided to 4 groups (n=10 in every group) and treated for 4 weeks by different treatments:

a. control (chow diet)
b. Rosiglitazone (chow diet + Rosiglitazone 0.02%)
c. Dunaliella (chow diet + crude Dunaliella powder 8%)
d. Rosiglitazone plus Dunaliella (chow diet + Rosiglitazone 0.02% + crude Dunaliella powder 8%).

8. The combination treatment of High-dose rosiglitazone plus crude Dunaliella powder did not improve the effect of High-dose rosiglitazone on diabetes in db/db mice (see fig. 3). In contrast, the combination treatment of Low-dose rosiglitazone plus crude Dunaliella powder had a beneficial effect on diabetes in db/db mice compared to low-dose rosiglitazone treatment alone (see Fig. 1a).

9. In summary, the addition of crude *Dunaliella* powder to treatment of diabetes with Rosiglitazone allows a lowering of the dose of the drug, thus reducing the adverse side-effects of Rosiglitazone.

10. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

10.2.05
Date

Aviv Shaish
Dr. Aviv Shaish